AMENDMENTS TO THE DRAWINGS

In the drawings:

The attached (5) sheets of drawings include changes to Fig. 1, 3-5. These (5) sheets replace the original (5) sheets comprising Figures 1-5.

Attachment: Replacement Sheets (5)

REMARKS

Claims 1-86 are pending in the application. Claims 26-31 are withdrawn. By this amendment, claims 2-13, 16-20, and 62-86 are canceled; and claims 1, 15, 32, 45, and 54 are amended. Accordingly, claims 1, 14, 15, and 21-61 are currently under examination. Support for the amendment of claim 1 is found in the specification, *inter alia*, on page 10, paragraph [0039]; page 13, paragraph [0049]; page 24, paragraph [0093]; and original claims 5 and 13. Support for the amendment of claim 15 is found in the specification, *inter alia*, on page 14, paragraph [0052]; and original claim 19. Claim 32 is amended to incorporate features in claim 31. Support for the amendment of paragraph [0011] is found in the specification, *inter alia*, on page 24, paragraph [0095]. Accordingly, no new matter has been added.

Figure 3 has been amended to include the description of Y axis. In addition, Figures 1 and 4-5 have been enlarged so as to comply with the drawing requirements. No new matter has been added.

With respect to all claim amendments and cancellations, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments in future continuation and/or divisional application.

Restriction/election

Applicants thank the Examiner for including claims 59-61 and 83-86 in Group I for examination.

Objections

Specification

The Examiner objects to the description of Fig. 1 on page 3, alleging that the abbreviation GSP is not expanded. Applicants respectfully note that the specification is amended to indicate that "GSP" refers to glycated serum proteins.

The Examiner objects to the description of Fig. 2, alleging it is not in proper idiomatic English. Applicants respectfully traverse this objection. Applicants note that it is clear to one skilled in the art that Figure 2 shows that the exemplary method has an assay linearity for a range of concentrations of the glycated protein in a sample. Applicants respectfully submit that the description of Figure 2 is proper.

The Examiner objects to the description of Figures 3 and 5, alleging that it does not describe what in Fig. 3 reacts with fructosyl valine and what signal is referred to in Fig. 5. Applicants respectfully traverse the objection. Applicants respectfully note that the Example 3 clearly describes the reaction shown in Figures 3 and 5. As described in Example 3, glycated valine was reacted with Proteinase K (in R2), and then with HRP and FAOD (in R3); and OD at 726nm was read. Thus, the description is clear for one skilled in the art in view of Example 3.

The Examiner further objects that the catalog number for Fructosamine Calibrator on page 27 lacks a name of the producer. Applicants respectfully note that the producer for the products is Diazyme as indicated on page 26, paragraph [0101].

In view of the above, Applicants respectfully request that the objections to the specification be withdrawn.

Figures

The Examiner objects to Figure 3, alleging that Figure 3 lacks description of Y-axis.

A replacement Figure 3 is submitted herewith, which contains a description of Y-axis.

Claims

Claim 32 is objected to for depending on nonelected claim 31. Applicants note that claim 32 is amended to incorporate features in claim 31. Accordingly, Applicants respectfully request that the objection be withdrawn.

Rejections under 35 U.S.C. §112, second paragraph

Claims 54 and 78 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner states that the claims are confusing in limiting inactivation of protease by heat currently with the presence of the chimeric protein. The Examiner further states that inactivation of protease by heat performed in the presence of chimeric protein is likely to inactivate the chimeric protein as well, making the method inoperative.

Without acquiescence to the objection, Applicants note that claim 78 is canceled, and claim 54 is amended to recite that the protease is inactivated by a heat treatment if the protease is inactivated before the contact between the glycated peptide or glycated amino acid and the chimeric protein. Applicants respectfully submit that claim 54 as amended is definite.

Claim 85 is rejected under 35 U.S.C. 112, second paragraph, as allegedly being confusing. The Examiner states that peroxidase will oxidize amadoriase preventing it from deglycating peptides and amino acids when amadoriase and peroxidase are in the same solution.

Applicants respectfully traverse this rejection. Applicants note that claim 85 is canceled; and thus, the rejection is addressed in view of the recitation in claim 61. Applicants respectfully

note that a peroxidase does not catalyzes an oxidizing reaction of amadoriase in the formulation. A peroxidase oxidizes a substrate by reducing hydrogen peroxide; and thus, in the absence of hydrogen peroxide, a peroxidase could not catalyze an oxidizing reaction. Thus, the chimeric protein and the peroxidase can be formulated in a single composition.

In view of the above, Applicants respectfully request that the rejections under 35 U.S.C. §112, second paragraph be withdrawn.

Rejections under 35 U.S.C. §112, first paragraph

Written description

Claims 1-10, 12, 14-24, 32-42, 45-66, 69-86 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner states that the claims are rejected because the structure or structure and function of the claimed product which is to be used in the claimed methods and kits lack sufficient written description. The Examiner further states that the claims are very broad as to the structure of the amadoriase and signal molecules used for construction of a chimeric protein, and the disclosure does not teach how to modify SEQ ID NO:3 so that a sequence having 40% homology was still having amadoriase activity. The Examiner further states that it is unknown what the function of SEQ ID NO:1 and 4 has to be so that the sequences having at least 40% of homology and retaining the function could be used for construction of the claimed chimeric proteins. The Examiner acknowledges that Applicants disclosed two species and methods of their use as well as kits for performing said methods, and these species are amadoriase of SEQ ID NO:3 having SEQ ID NO:1 attached to its N-terminus, and protein of SEQ ID NO:5 consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:4, however, the Examiner alleges that neither of these species is sufficient to identify a broad genus of chimeric proteins claimed by the Applicants. The Examiner concludes that one skilled in the art is not convinced that Applicants were in possession of the claimed invention at the time the application was filed because the structure and function of the claimed genus of products is not sufficiently disclosed i.e., and because the claims are lacking sufficient written description of

structure and function. The Examiner further notes that the structural limitations recited by claim 12 are so minor that the scope of the claimed proteins covers many proteins with virtually no structural homology to SEQ ID NO:3 at all.

Without acquiescence to the rejections and in the interest of expediting prosecution, Applicants have amended claim 1 to recite that the chimeric protein has the enzymatic activity of an amadoriase, the leader sequence comprises the amino acid sequences set forth in SEQ ID NO:1, and the amadoriase comprises the amino acid sequence set forth in SEQ ID NO:3. In view of the claim amendment, Applicants respectfully submit that written description is satisfied by functional characteristics coupled with a known or a disclosed correlation between function and structure. Claims as amended recites the chimeric protein comprising specific sequences with a known function. Applicants respectfully submit that claims as amended comply with the written description requirement.

In view of the above, Applicants respectfully request that the rejection be withdrawn.

Enablement

Claims 1-12, 14-24, 32-42, 45-66, 69-86 are rejected, as allegedly not being enabling for the scope of the claims. The Examiner states that because the specification, while being enabling for the chimeric protein consisting of SEQ ID NO:1 attached to N-terminus of SEQ ID NO:3, or for chimeric protein identified by SEQ ID NO:5 as well as the methods of their use and kits for said methods, does not reasonably provide enablement for any chimeric protein comprising: 1) any amadoriase, including a protein having at least 40 % identity to SEQ ID NO:3, 2) any peptidyl leader that is 40% identical to SEQ ID NO: 1 or 4 and any amadoriase, and 3) any bacterial leader, any amadoriase and any second bacterial leader, as well as methods of use of said chimeric protein use and kits for the methods.

Without acquiescence to the rejections and in the interest of expediting prosecution, Applicants have amended claim 1 to recite that recite that the chimeric protein has the enzymatic activity of an amadoriase, the leader sequence comprises the amino acid sequences set forth in SEQ

ID NO:1, and the amadoriase comprises the amino acid sequence set forth in SEQ ID NO:3. Applicants respectfully submit that claims as amended are enabled. One skilled in the art can make and use the chimeric protein comprising the specific sequences as recited in the claims as amended without undue experimentation in view of the ample guidance provided in the specification. Methods for assaying the enzymatic activity of an amadoriase is known in the art and described in the specification. *See, e.g.*, pages 11-13, paragraphs [0043]-[0051]. The specification also teaches methods of using the chimeric protein for assaying a glycated protein in a sample. *See, e.g.*, pages 18-21, paragraphs [0062]-[0077]; and Examples. Thus, Applicants respectfully submit that claims as amended are enabled.

The Examiner further notes that claims 45 and 69 are not enabled because there are no known methods for measuring changes in amount of H_2O in an aqueous assays as changes in amount of water caused by deglycation is negligible compared to the amount of water present in the reaction vessel.

Without acquiescence to the rejection, Applicants note that claim 45 is amended to delete the term "H_2O", and claim 69 is canceled.

In view of the above, Applicants respectfully request the rejections be withdrawn.

Rejections under 35 U.S.C. §103

A. Claims 1-2, 6-14, 21-22 and 32 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Yoshida et al. (Eur. Biochem. 1996, 242, 499-5050), Takahashi et al. **A** (J. Biol. Chem., 1997, 6:12505-12307) in view of common knowledge in the field of protein expression as exemplified by US Patent 6,194,200. The Examiner states that Yoshida et al teach the use of several amadoriases, among them of *Aspergillus* species, for determination of the level of blood protein glycation in diabetic patients. The Examiner states that Takahashi et al. teach amadoriase from Aspergillus sp. that consists of 438 amino acid residues of which amino acids 2-438 are identical to amino acids 1-437 of SEQ ID NO:3 of the instant application, and Takahashi et al. do not disclose a chimeric protein, which comprises a bacterial leader sequence and an

amadoriase, however, it is a routine practice in the field of protein expression to express proteins as chimeras containing bacterial leader sequences because these sequences promote secretion and stabilize the expressed protein; see US Patent 6,194,200, Introduction. The Examiner concludes that it would have been obvious to one having ordinary skill in the art at the time of invention to have a protein consisting of amino acid residues 2-438 of Takahashi et al. amadoriase II and add to its N-terminus a bacterial leader as taught by the US Patent 6,194,200.

Without acquiescence to the rejections and in the interest of expediting prosecution, Applicants has amended claim 1 to incorporate features in claims 5 and 13, and claims 14, 15, 21-25, and 32 depend from claim 1. Claim 5 is not rejected by the Examiner as being obvious over the references.

Applicants respectfully submit that the references cited by the Examiner, even if combined, do not teach or suggest all the claim limitations as amended. The references cited by the Examiner, even if combined, do not teach or suggest that a chimeric protein comprising, from N-terminus to C-terminus, a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues, wherein the leader sequence comprises the amino acid sequence set forth in SEQ ID NO:1; and a second peptidyl fragment comprising an amadoriase comprising the amino acid sequence set forth in SEQ ID NO:3. Since the references cited by the Examiner do not teach or suggest all the claim limitations as amended, the Examiner has not established a *prima facie* case of obviousness. Applicants respectfully request that the rejection be withdrawn.

B. Claims 33-37, 39-41, 45, 53, 54, 57, 58, 59-61 are rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over European Patent EPO 821064 A2, and further in view of Takahashi et al. A and US Patent 6,194,200 as used in the above rejection of the chimeric protein of claim 1. The Examiner states that the EU Patent discloses in Examples 6 and 7, page 16, a method of measurement of the level of glycation of a protein, namely of serum albumin and hemoglobin, which consists of the same steps as those of claim 33, and the method uses fructosyl amino acid oxidase (amadoriase) from *Aspergillus terreus*. The Examiner concludes that it would have been obvious to one having ordinary skills in the art to modify teachings of the EU Patent and replace

fructosyl amino acid oxidase (amadoriase) from *Aspergillus terreus* with the chimeric protein of the invention.

Without acquiescence to the rejections and in the interest of expediting prosecution, Applicants has amended claim 1 to incorporate features in claims 5 and 13, and claims 33-37, 39-41, 45, 53, 54, and 57-61 recite all features in claim 1.

Applicants respectfully submit that the references cited by the Examiner, even if combined, do not teach or suggest all the claim limitations as amended. The references cited by the Examiner, even if combined, do not teach or suggest that a method and a kit for assaying a glycated protein using a chimeric protein comprising, from N-terminus to C-terminus, a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues, wherein the leader sequence comprises the amino acid sequence set forth in SEQ ID NO:1; and a second peptidyl fragment comprising an amadoriase comprising the amino acid sequence set forth in SEQ ID NO:3. Since the references cited by the Examiner do not teach or suggest all the claim limitations as amended, the Examiner has not established a prima facie case of obviousness. Applicants respectfully request that the rejection be withdrawn.

C. Claims 62-63, 65, 66, 83 and 85 are rejected as allegedly being unpatentable over European Patent EPO 821064 A2 in view of common knowledge in biochemistry and Takahashi et al. **B** (J. Biol. Chem., 1007, 272, 3437-3443).

Applicants note that claims 62, 63, 65, 66, 83, and 85 have been canceled. Thus, this rejection is moot. Applicants respectfully request the rejection be withdrawn.

In view of the above, Applicants respectfully request that the rejections under 35 U.S.C. §103 be withdrawn.

Conclusion

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 466992001300. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: November 22, 2005

Respectfully submitted,

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